Formation of Quaternary Pyridinium Compounds by the Action of Glutaraldehyde on Proteins

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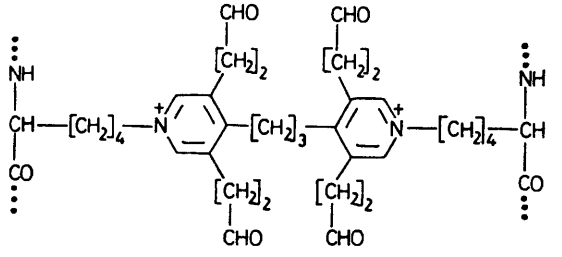
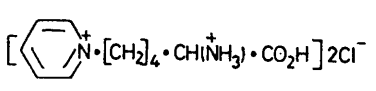
Summary 1-(5-Amino-5-carboxypentyl)pyridinium chloride hydrochloride (**2**) has been isolated by acid hydrolysis of the product of the action of glutaraldehyde on ovalbumin, supporting the view that the chromophore with λ_{max} *ca.* 265 nm formed when glutaraldehyde reacts with proteins is due to the formation of quaternary pyridinium compounds.

ON the basis of model experiments with glutaraldehyde and ϵ-aminocaproic acid and α-acetyl-lysine, it has been suggested[1] that the cross-linking action of glutaraldehyde on proteins is due to the formation of structures such as (**1**); the chromophore at λ_{max} *ca.* 265 nm which develops during the cross-linking reaction is ascribed to the quaternary pyridinium system present in (**1**). We now present more direct evidence in support of this hypothesis.

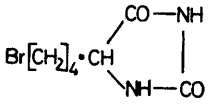
Ovalbumin was stirred for 90 min with glutaraldehyde (12·5% w/v) in 0·2 M ammonium acetate buffer (pH 4·7). After dialysis the modified protein, an amino-acid analysis of which showed that 50% of the lysine residues had been modified, was oxidised with performic acid and the oxidised product hydrolysed with 6 M HCl at 110 °C for 64 h. Chromatography of the hydrolysate on a variety of columns gave several products with λ_{max} *ca.* 260 nm; one of these was also present when the oxidation procedure was replaced by a Strecker synthesis[2] and when the reaction product was directly hydrolysed without any treatment to modify residual aldehydo-groups.



(1)

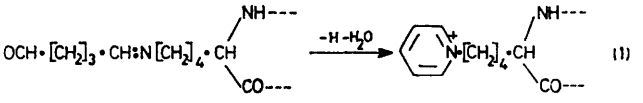


(2)



(3)

The product common to all three procedures was identified as 1-(5-amino-5-carboxypentyl)pyridinium chloride hydrochloride (**2**) by direct comparison with a specimen synthesised from the bromobutylhydantoin (**3**)[3] by heating under reflux with pyridine, followed by hydrolysis of the resulting pyridinium hydantoin with 6 M HCl. A satisfactory elemental analysis was obtained for the synthetic product. The identity of this with the product from ovalbumin was established by t.l.c. (4 solvent systems on silica gel, 4 solvent systems on cellulose) and from their u.v. (λ_{max} 254, 257, and 265 nm in H_2O) and 100 MHz ^1H n.m.r. [τ(CF_3CO_2D) 8·94 (2H, d, pyridine 2- and 6-H), 8·74 (1H, t, pyridine 4-H), 8·26 (2H, t, pyridine 3- and 5-H), 4·80 (2H, t, ϵ-CH_2), 4·50 (1H, m, α-CH), and 2·28 and 1·86 (6H, m, β-, γ-, and δ-CH_2)] spectra.



(4)

The isolation of the pyridinium salt (**2**) confirms our view[1] that the chromophore at *ca.* 265 nm in the reaction products of glutaraldehyde with proteins is due to quaternary pyridinium compounds. The simple compound (**2**) will arise by the oxidative cyclisation, in the unhydrolysed protein, of the Schiff s base (**4**) as shown in reaction (1). The Schiff's base (**4**) is the first step on the way to more complex structures, such as (**1**), by further reaction with lysine side-chains and glutaraldehyde molecules.

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[2] P. Bolivar, M. A. Paz, P. M. Gallop, and O. O. Blumenfeld, *Biochem. Biophys. Res. Comm.*, 1973, **55**, 96.
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